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How multiplex testing approach to respiratory viruses detection can enhance influenza surveillance

Elena Pariani[a,b,1], Renèe Pasciuta[c,1], Federica Novazzi[c], Andreina Baj[c,d,*], Cristina Galli[a], Laura Pellegrinelli[a], Laura Bubba[a], Danilo Cereda[e], Simona Puzelli[f], Daniele Focosi[g], Massimo Agosti[d,h], Paola Stefanelli[f], Fabrizio Maggi[c,d]

[a] Department of Biomedical Sciences for Health, University of Milan, Varese, Italy
[b] Inter-University Centre for Research on Influenza and Other Communicable Infectious Diseases (CIRI IT), University of Genoa, Italy
[c] Laboratory of Microbiology, ASST Sette Laghi, Varese, Italy
[d] Department of Medicine and Surgery, University of Insubria, Varese, Italy
[e] Lombardy Region, Directorate-General for Health, UO Prevenzione, Milan, Italy
[f] Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy
[g] North-Western Tuscany Blood Bank, Pisa University Hospital, Pisa, Italy
[h] Department of Neonatology, Neonatal Intensive Care Unit, and Pediatrics, F. Del Ponte Hospital, University of Insubria, Varese, Italy

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Dear Editor,

We report an autochthonous case of influenza A subtype H3N2 infection in a child from Northern Italy, who developed influenza-like illness (ILI) on September 25, 2021. This was the first laboratory-confirmed case of influenza in Italy since March 2020. Although the national sentinel ILI surveillance system (InfluNet, coordinated by the Italian National Institute of Health) was maintained during the SARS-CoV-2 pandemic, no case of influenza infections has been reported for more than one year and a half [1]. Since the COVID-19 pandemic upsurge, influenza activity has dramatically decreased with a very little circulation of influenza viruses [2,1]. In recent times, sporadic detection of the influenza virus has been reported in Europe [3].

On September 26, 2021, a child was admitted to the pediatric emergency hospital, with respiratory distress, fever (38 °C), sore throat, runny nose, and cough. After clinical evaluation and according to the current Italian Ministry of Health and Lombardy Region SARS-CoV-2 surveillance protocols, a nasopharyngeal swab (NPS) was collected and analyzed with a multiplex real-time reverse transcription-polymerase chain reaction (RT-PCR) test intended for the qualitative and simultaneous detection and differentiation of influenza virus A, influenza virus B, respiratory syncytial virus, and SARS-CoV-2 (Alinity m Resp-4-Plex assay, Abbott).

Unexpectedly, the NPS tested positive for influenza A virus (cycle threshold 18) and negative to the other investigated targets. The diagnosis of laboratory-confirmed influenza infection was made, and the patient was dismissed the same day in good clinical conditions.

As part of the regional Influenza Surveillance Plan in the Lombardy region and the national sentinel surveillance system for influenza syndromes (InfluNet), the influenza A subtype was characterized by the regional reference laboratory (Department of Biomedical Sciences for Health, University of Milan) using a real-time RT-PCR assay targeting the hemagglutinin (HA) gene as per the CDC protocol [4]. Influenza virus A(H3) subtype was identified and the se-

Abbreviations: ILI, influenza-like illness; SARS-CoV-2, SARS coronavirus 2.

* Corresponding author at: Department of Medicine and Surgery, University of Insubria, Varese, Italy.
E-mail address: andreina.baj@uninsubria.it (A. Baj).

[1] These authors contributed equally to this work.

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quence analysis of the HA [5,6] showed that the virus belonged to 3C.2a clade, sharing a nucleotide identity of 96.9% with the reference strain A/Hong Kong/5738/2014 (deposited in GISAID, accession number EPI ISL 5,159,327). This strain segregated into the subclade 3C.2a1, group 3C.2a1b, and subgroup T131K-A, sharing a nucleotide identity of 98.5% and an amino acid similarity of 98.1% with the reference strain A/Cambodia/e0826360/2020, selected as A(H3N2) vaccine strain for the Northern Hemisphere for the upcoming 2021–2022 season.

In conclusion, the first evidence of a laboratory-confirmed influenza virus infection in Italy clearly shows the importance of implementing a multiplex testing approach to respiratory infections to detect the increasing influenza surveillance granularity. The SARS-CoV-2 pandemic has increased awareness of the dramatic impact that respiratory infections may have on human health and has given the unprecedented opportunity to establish a network of laboratories with increased expertise in the molecular diagnosis of respiratory agents. This may also justify the need of testing all patients admitted to the emergency room with respiratory infections in a current protocol of influenza surveillance to increase its sensitivity and the ability to catch in real-time the introduction and circulation of influenza viruses.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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